

Studies on Phytochemicals, Antioxidant and Anticancer Potentials of *Evolvulus Alsinoïdes* using Lung Cancer Cell Line

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ABSTRACT

The main objective of this study was to evaluate the phytochemicals, antioxidant potentials and anticancer property of *Evolvulus alsinoides*. Initially, plant extract was prepared using different solvents includes petroleum ether, ethanol and ethyl acetate. The antioxidant potential of the selected plant extract was assessed by Super oxide radical assay, Hydroxyl radical assay and DPPH assays and the results were analyzed. The anticancer property was observed against lung cancer cell line, A-549. From the results it was found that, Alkaloids, Steroids, Flavonoids, Tannins/Phenol, Proteins, Carbohydrate and Glycosides are present in the ethanolic extract of the plant. No significant compounds could be detected in other extracts including petroleum ether and ethyl acetate. DPPH assay and Ferric thiocyanate method clearly reveals that, this plant extract has significant antioxidant potential in dose dependent manner. It also showed notable inhibitory potential against the growth of the lung cancer cells.

KEYWORDS: phytochemicals, antioxidant, anticancer, lung cancer

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INTRODUCTION

Medicinal plants are found to be the unavoidable sources to explore novel drugs against wide range communicable and non-communicable diseases for the past few centuries (Mohanasundaram *et al.*, 2016). Drugs against many diseases including cancer, diabetes, arthritis are being developed from plant sources. Cancer has existed for all of human history. The earliest written record regarding cancer is from circa 1600 BC in the Egyptian Edwin Smith Papyrus and describes cancer of the breast. Medically it is known as a malignant neoplasm (Hockeberry *et al.*, 1993). Cancer is a broad group of diseases involving unregulated cell growth in which cells divide and grow uncontrollably, forming malignant tumors, and invading nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous. Benign tumors do not invade neighbouring tissues and do not spread throughout the body (Hockenberry *et al.*, 1990). There are over 200 different known cancers that affect humans.

Discovery of effective herbs and elucidation of their underlying mechanisms could lead to development of an alternative and complimentary method for cancer prevention and treatment. The mechanism of inhibition of tumour progression by natural phytochemicals range from inhibition of genotoxic effects, increased anti-inflammatory and antioxidant effect, inhibition of cell proliferation,

protection of intracellular communications to modulate apoptosis and signal transduction pathways.

The crude extracts of *Emblica officinalis* and *Evolvulus alsinoides* were evaluated for immunomodulator activity in adjuvant induced arthritic rat model. The anti-inflammatory response of both the extracts was determined by lymphocyte proliferation activity and histopathological severity of synovial hyperplasia. Both the extracts showed a marked reduction in inflammation and edema. At cellular level immunosuppression occurred during the early phase of the disease. There was mild synovial hyperplasia and infiltration of few mononuclear cells in *E. officinalis* or *Evolvulus alsinoides* treated animals. The induction of nitric oxide synthase (NOS) was significantly decreased in treated animals as compared to controls (Roukos DH, 2009).

The extract of *E. alsinoides* showed dose dependent effects in controlling the diarrhoea. The results are similar to those of the standard drug diphenoxylate with regard to severity of diarrhea (Croce CM, 2008). The in vivo evaluation of the alcoholic extract of *Evolvulus alsinoides* revealed its marked antiulcer and antitumor activity (Henderson *et al.*, 2000).

Hence, the main objective of this study was to evaluate the anticancer potential of this plant against lung cancer cell lines along with evaluation of antioxidant capacity and the major phytochemicals found in it.

Materials and Methods

Plant Material

The fresh sample of the plant was collected and authenticated. The fresh leaves were washed in running tap water and shade dried for one week in the laboratory conditions. The dried sample was ground into fine powder using mechanical grinder. Different solvents, petroleum ether, ethanol and ethyl acetate were used for the extraction using soxhlet apparatus.

Phytochemical analysis

The major phytochemical present in the plant sample in all the three solvents were analyzed using the standard proven procedure (Harbone, 1998, VA Doss et al., 2016).

Antioxidant and anticancer properties

The suspected antioxidant potential was assessed by DPPH assay, Super oxide radical assay and Hydroxyl radical assay using the standard proven methods (Mohanasundaram et al., 2019). The invitro anticancer property was studied by MTT assay using lung cancer cell line, A-549 (Mohanasundaram et al., 2019).

Results and Discussion

Table 1 Phytochemical analysis of *Evolvulus alsinoides*

Compound	Petroleum ether	Ethanol	Ethyl acetate
Alkaloids	+	+	--
Steroids	+	+	+
Flavanoids	--	+	+
Tannins/Phenol	--	+	--
Proteins	--	+	--
Carbohydrate	+	+	--
Glycosides	+	+	+
Terpenoids	--	--	+

"+" – Present

"-" – Absent

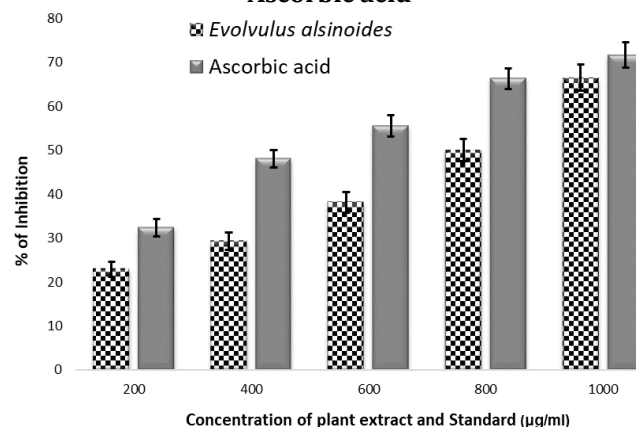
The qualitative phytochemical analysis of the selected plant using different solvents were analyzed. It showed (Table 1) very clearly that the ethanolic extract was found to be best and containing most of the phytochemicals. Hence, the ethanolic extract was chosen for further analysis.

Antioxidant Assays

DPPH Assay

The results of DPPH assay (Fig. 1) notably exemplify that the ethanolic extract of our plant showed significant scavenging activity against DPPH radicals and the results obtained are almost similar to that of earlier study (Mohanasundaram et al., 2019). At 1000mg, ethanolic extract showed 66.42% of inhibition DPPH radical generation whereas standard showed 71.65% of inhibition. Hence, this extract could be potential therapeutic choice against diseases like cancer where extensive cell damages are witnessed.

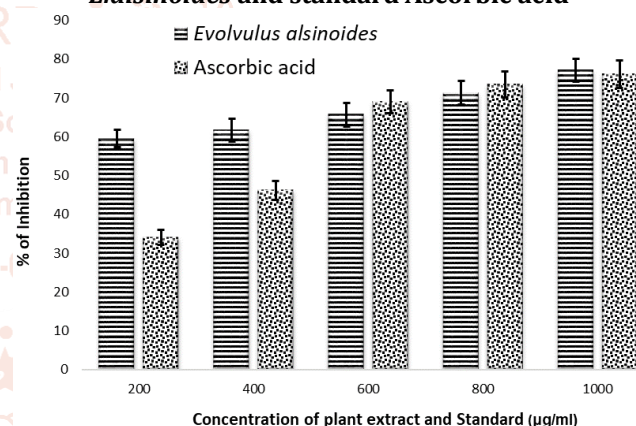
Figure 1 DPPH assay of *E.alsinoides* and standard Ascorbic acid



Superoxide Radical Scavenging Assay

The Superoxide Radical Scavenging assay is an another supporting evidence (Fig.2) for the antioxidant potential of the ethanolic extract of our plant. Similar to that of DPPH assay, the antioxidant potential of the extract has been witnessed as dose dependent. The results are similar and comparable to earlier study (Mohanasundaram et al., 2019). At 1000mg, ethanolic extract showed 77.06% of inhibition DPPH radical generation whereas standard showed 76.11% of inhibition.

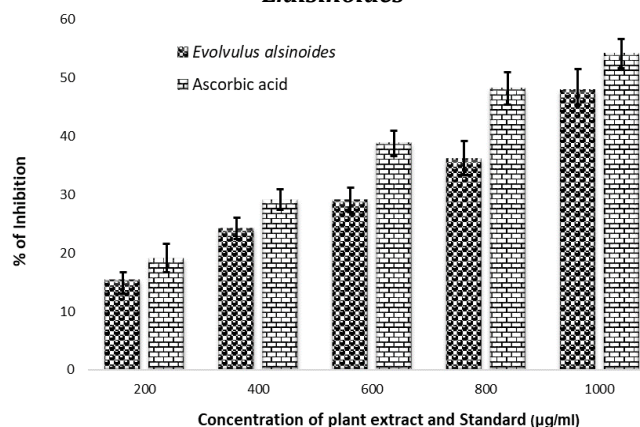
Figure 2 Superoxide radical scavenging assay of *E.alsinoides* and standard Ascorbic acid



Hydroxyl radical scavenging assay

The ethanolic extract of *E.alsinoides* showed (Fig. 3) 47.88% inhibition of hydroxyl radical generation and the standard showed 54.12% of inhibition. The gradual increase in the antioxidant potential has been witnessed when the concentration increases from 200 to 1000mg in all the antioxidant assays.

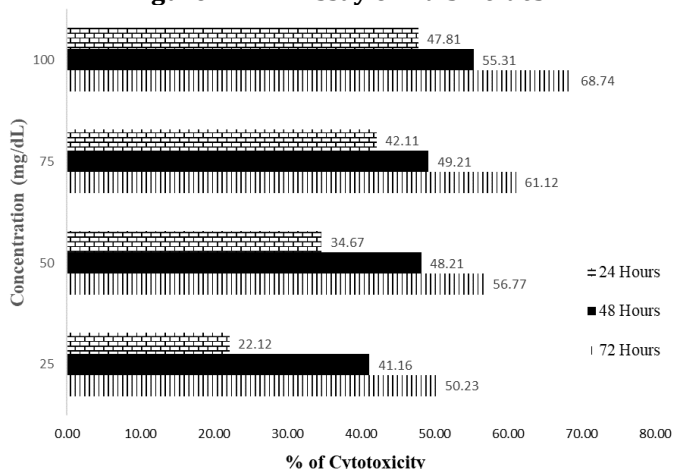
Figure 3 Hydroxyl Radical Scavenging Assay of *E.alsinoides*



Cytotoxicity Assay (MTT Assay)

The MTT assay showed (Figure 4) the possible cytotoxic potential of the ethanolic extract of the *E.alsinoides* against the growth of lung cancer cell line, A-459. The gradual increase in the cytotoxicity was observed when the concentration of the plant extract increases from 25mg to 50, 75 and 100mg.

Figure 4 MTT Assay of *E.alsinoides*



From the results, we have observed around 68.74% of inhibition after 72Hr at the concentration of 100mg/dL. In the same duration, 55.31% and 47.81% of inhibition of cancer growth has also been witnessed at the concentration of 100mg/dL after 48 and 24Hrs respectively. The results are comparable with earlier study (Mohanasundaram et al., 2019), and hence, the extract can be potential choice of therapy against cancer.

It has been proven in so many studies that, antioxidants can be used as a therapeutic option to eradicate major illness including cancer. When, any natural found to contain both antioxidant and cytotoxic potentials are undoubtedly a best option to use against wide range of diseases.

Conclusion

The ethanolic extract of *E.alsinoides* was found to contains the major phytochemicals like alkaloids, phenols, flavanoids, saponins, etc. The antioxidant assays of the ethanolic extract showed its huge potential against wide range of free radical generation and in addition to this, it has also been characterized with huge cytotoxicity against the growth of lung cancer cell line. Hence, this extract should be studied

thoroughly to isolate the exact compounds being responsible for the suspected bioactivities and hence further studies are warranted.

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